

What is claimed is:

1. A polypeptide cleavage method characterized in that arginine or lysine is at the P1 position of a desired cleavage site in a polypeptide, an amino acid
5 other than aspartic acid, glutamic acid or proline is at the P1' position, a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5'
10 position (with the proviso that a single basic amino acid is not situated at the P6 or P4 position), and OmpT protease is used to cleave the desired cleavage site in said polypeptide.

2. A method for producing a target peptide
15 characterized by obtaining a target peptide from a fusion protein, the cleavage site of the fusion protein being a desired cleavage site comprising a protecting peptide whose C-terminus is arginine or lysine, fused via the desired cleavage site with a target peptide whose N-
20 terminus is an amino acid other than aspartic acid, glutamic acid or proline, wherein a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position
25 to the P5' position (with the proviso that in the case of a single basic amino acid, it is not situated at the P6 or P4 position), host cells are transformed with an expression plasmid having a gene coding for the fusion protein wherein said cleavage site is a cleavage site
30 which is cleavable by OmpT protease, and said gene is expressed in said cells and is cleaved by said protease at said cleavage site.

3. The method of claim 1 or 2 wherein, if a site which is not desired to be cleaved by OmpT protease is
35 present in the polypeptide or the fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

4. The method of any one of claims 1 to 3, which comprises situating two or three consecutive basic amino acids between the P10 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

5 5. The method of claim 4, which comprises situating three consecutive basic amino acids between the P5 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

10 6. The method of any one of claims 1 to 5, wherein the basic amino acids are arginine and/or lysine.

7. The method of 6, wherein the basic amino acids are arginine.

15 8. A polypeptide cleavage method wherein OmpT protease is used for cleavage at a desired cleavage site in the polypeptide, or a method for producing a target peptide which comprises cleavage at a desired cleavage site in a fusion protein, the method being characterized in that, if a site which is not desired to be cleaved by OmpT protease is present in said polypeptide or said
20 fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

9. The method of any one of claims 3 to 8, wherein the acidic amino acid is aspartic acid.

25 10. The method of any one of claims 1 to 9, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Arg-Arg-Arg-Ala-Arg.

30 11. The method of any one of claims 1 to 9, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Asp-Ala-Arg-Arg-Arg-Ala-Arg.

35 12. A polypeptide cleavage method characterized by cleaving a desired cleavage site of a polypeptide using an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine,

threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

13. A polypeptide cleavage method characterized in that, when the P1 position of the desired cleavage site in the polypeptide is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, the desired cleavage site of said polypeptide is cleaved using an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

14. A polypeptide cleavage method characterized in that the P1 position of the desired cleavage site in the polypeptide is arginine or lysine, the P1' position is an amino acid other than arginine or lysine, a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position, and the desired cleavage site of said polypeptide is cleaved using an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

15. A method for producing a target peptide, characterized by transforming host cells with an expression plasmid having a gene coding for a fusion protein comprising a target peptide fused with a protecting peptide via a desired cleavage site that can be cleaved by an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from

the fusion protein by cleavage with said protease at said cleavage site.

16. A method for producing a target peptide, characterized by transforming host cells with an expression plasmid having a gene coding for a fusion protein comprising a protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via a desired cleavage site that can be cleaved by an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.

17. A method for producing a target peptide, characterized by transforming host cells with an expression plasmid having a gene coding for a fusion protein wherein a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position at a desired cleavage site of a fusion protein comprising a protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via the cleavage site, and said desired cleavage site is a cleavage site that can be cleaved by an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.

18. The method of any one of claims 12 to 17

wherein, if a site which is not desired to be cleaved by the OmpT protease 97th amino acid variant is present in the polypeptide or fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

19. The method of any one of claims 12 to 18, which comprises situating two or three consecutive basic amino acids between the P10 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

20. The method of claim 19, which comprises situating three consecutive basic amino acids between the P5 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

21. The method of any one of claims 14 or 17 to 20, wherein the basic amino acids are arginine and/or lysine.

22. The method of claim 21, wherein the basic amino acids are arginine.

23. A polypeptide cleavage method wherein an OmpT protease 97th amino acid variant is used for cleavage at a desired cleavage site in the polypeptide, or a method for producing a target peptide which comprises cleavage at a desired cleavage site in a fusion protein, the method being characterized in that, if a site which is not desired to be cleaved by the OmpT protease 97th amino acid variant is present in said polypeptide or said fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

24. The method of any one of claims 18 to 23, wherein the acidic amino acid is aspartic acid.

25. The method of any one of claims 12 to 24, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Arg-Arg-Arg-Ala-Arg.

26. The method of any one of claims 12 to 24, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide

or fusion protein is Asp-Ala-Arg-Arg-Arg-Ala-Arg.

27. The method of any one of claims 12 to 26, wherein the 97th amino acid from the N-terminus of the OmpT protease is leucine, methionine or histidine.

5 28. The method of any one of claims 12 to 26, wherein the P1' position of the desired cleavage site of the polypeptide or fusion protein or the N-terminus of the target peptide is serine or alanine, and the 97th amino acid of the OmpT protease 97th amino acid variant
10 used is leucine.

29. The method of any one of claims 12 to 26, wherein the P1' position of the desired cleavage site of the polypeptide or fusion protein or the N-terminus of the target peptide is phenylalanine, alanine, serine,
15 cysteine or tyrosine, and the 97th amino acid of the OmpT protease 97th amino acid variant used is methionine.

30. The method of any one of claims 12 to 26, wherein the P1' position of the desired cleavage site of the polypeptide or fusion protein or the N-terminus of
20 the target peptide is alanine, valine, isoleucine, methionine, serine, threonine, cysteine or asparagine, and the 97th amino acid of the OmpT protease 97th amino acid variant used is histidine.

31. The method of any one of claims 2 to 11 and 15
25 to 30, wherein the target peptide is a peptide composed of between 22 and 45 amino acid residues.

32. The method of claim 31, wherein the target peptide is adrenocorticotrophic hormone (1-24), motilin or calcitonin precursor.

30 33. The method of any one of claims 2 to 11 and 15 to 32, wherein the host cells are *E. coli*.

34. The method of any one of claims 1 to 33, which comprises using as the cleaving protease bacterial cells expressing a gene coding for OmpT protease or an OmpT
35 protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine,

cysteine, asparagine, glutamine, glutamic acid or histidine.

35. The method of any one of claims 1 to 33, which comprises co-expressing a gene coding for OmpT protease or an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, and a gene coding for a polypeptide or fusion protein whose cleavage by said protease is desired.